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Inventor Information for 09/674815

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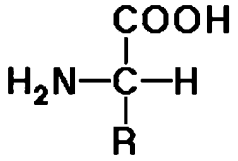
Chem 336 - Spring 1999 - Organic Chemistry III
Portland State University - Dr. Carl C. Wamser

Chapter 27 - Proteins

Amino Acids

- **natural amino acids are alpha (position of the amine) and L (stereochemistry)**



- **structures differ by the R groups**
  there are 20 common natural amino acids
  10 amino acids are essential to human nutrition
  (we can biosynthesize the others)

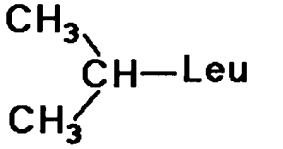
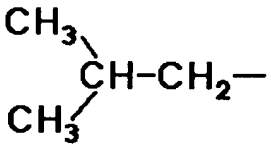
Side Chains

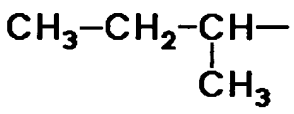
- **include a great variety of functional groups and properties**
- **glycine has no side chain**

Gly glycine $H_2N\text{-}CH_2\text{-}COOH$

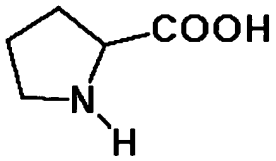
- **simple alkyl groups (nonpolar)**

Ala alanine R = CH_3-

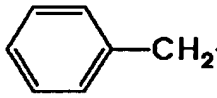
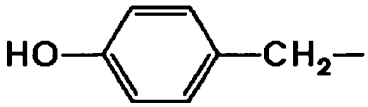
Val valine  leucine 

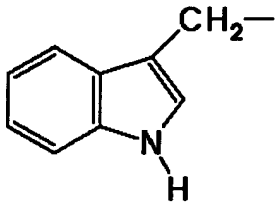
Ile isoleucine 

- **cyclic side chain (a secondary amine)**

Pro proline 

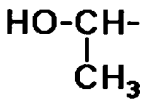
- **aromatic side chains**

Phe phenylalanine  Tyr tyrosine 

Trp tryptophane 

- **alcohol side chains**

Ser serine $HO\text{-}CH_2\text{-}$

Thr threonine 

(also note that tyrosine is a phenol)

- **sulfur side chains**

Cys cysteine $HS\text{-}CH_2\text{-}$ Met methionine $CH_3\text{-}S\text{-}CH_2\text{-}CH_2\text{-}$

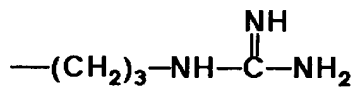
- **carboxylic acid side chains**

Asp aspartic acid $—CH_2—COOH$ Glu glutamic acid $—CH_2—CH_2—COOH$

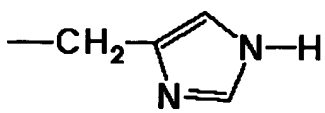
- **amide side chains (from the above acids)**

Asn asparagine $—CH_2—CONH_2$ Gln glutamine $—CH_2—CH_2—CONH_2$

- **basic side chains**

Arg arginine 
(guanidine group)

Lys lysine $—(CH_2)_4—NH_2$

His histidine 

Acid-Base Properties of the Amino Acids

- **basic amine group and acidic carboxyl group are typically both in their ionized forms in aqueous solution around biological pH values**
- **pKa for the alpha-amino group is about 9-10**
- **pKa for the carboxylic acid group is about 2-3**
- **below pH ~ 2, mainly cationic form (ammonium ion)**
- **between pH ~ 2 - 10, mainly zwitterionic form (both ammonium cation and carboxylate anion)**
- **above pH ~ 10, mainly anionic form (carboxylate anion)**
- **amino acids with side chains that can also ionize have more complicated behavior**

The Isoelectric Point

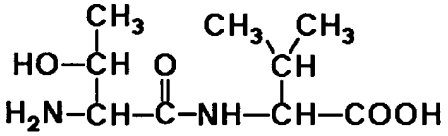
- **pH at which the major form of the molecule is neutral (and there are equal - but small - amounts of cationic and anionic forms)**
- **for most amino acids, the isoelectric point is midway between their two pKa values (the middle of their neutral range)**
- **for amino acids with ionizable side chains, isoelectric points are at higher pH (for basic side chains) and lower pH (for acidic side chains)**

Electrophoresis

- **amino acids can be separated based on their charge in solution at a given pH e.g., at pH 7, alanine is aprox. neutral, arginine is mainly +, glutamic acid is mainly -**
- **depending on their charge, the molecules migrate towards either a positive or negative electrode (moving through wet gel or paper)**

Peptides - Amino Acids joined by Amide Bonds

- **naming goes from N-terminus (free amine) to C-terminus (free carboxyl)**
- **-ine ending of the amino acids are replaced by -yl (except the last one) e.g., threonylvaline is a dipeptide**



Thr-Val (threonylvaline)

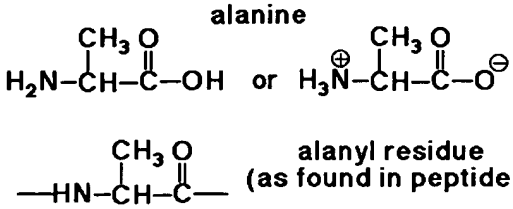
- **note that valylthreonine would be a different dipeptide**

Polypeptides

- **peptides are abbreviated with 3-letter codes (sometimes 1-letter codes)**
- **note the huge variety of possible polypeptides, using just the 20 common amino acids as building blocks**
  20 amino acids
  400 dipeptides
  8,000 tripeptides
  160,000 tetrapeptides
- **typical proteins have 100 or more amino acids, so the variety is immense**
- **note also the variety of possible functional groups available in those 20 amino acids**
- **peptides have evolved with a complete array of properties useful for life processes**

Amino Acid Residues

- **an amino acid residue differs from an amino acid by H_2O**



Disulfide Linkages

- **cysteine is readily crosslinked to another cysteine by a S-S bond**

- **disulfide linkages can hold together parts of a peptide chain that are not necessarily close in the peptide sequence**

Amino Acid Analysis

- **primary structure - the order of amino acids in the peptide chain**
- **complete hydrolysis (extended heating in aqueous HCl) breaks all amide bonds and releases all the individual amino acids**
- **individual amino acids can be separated by electrophoresis and/or chromatography and detected with ninhydrin (purple spot appears on reaction with any alpha-amino acid)**
- **complete hydrolysis, separation, and ninhydrin analysis can give a count of all amino acids and their relative abundance in the peptide**

Partial Hydrolysis of Peptides

- **occasionally, partial hydrolysis is carried out and smaller peptides are isolated, which are easier to analyze completely**
- **from the smaller peptides, sometimes useful connection information can be determined**
- **chymotrypsin and trypsin (digestive enzymes) are often used because they cleave peptides selectively**
  - **chymotrypsin cleaves a peptide only on the carboxyl side of the aromatic amino acids (Phe, Tyr, Trp)**
  - **trypsin cleaves a peptide only on the carboxyl side of the strongly basic amino acids (Lys and Arg)**

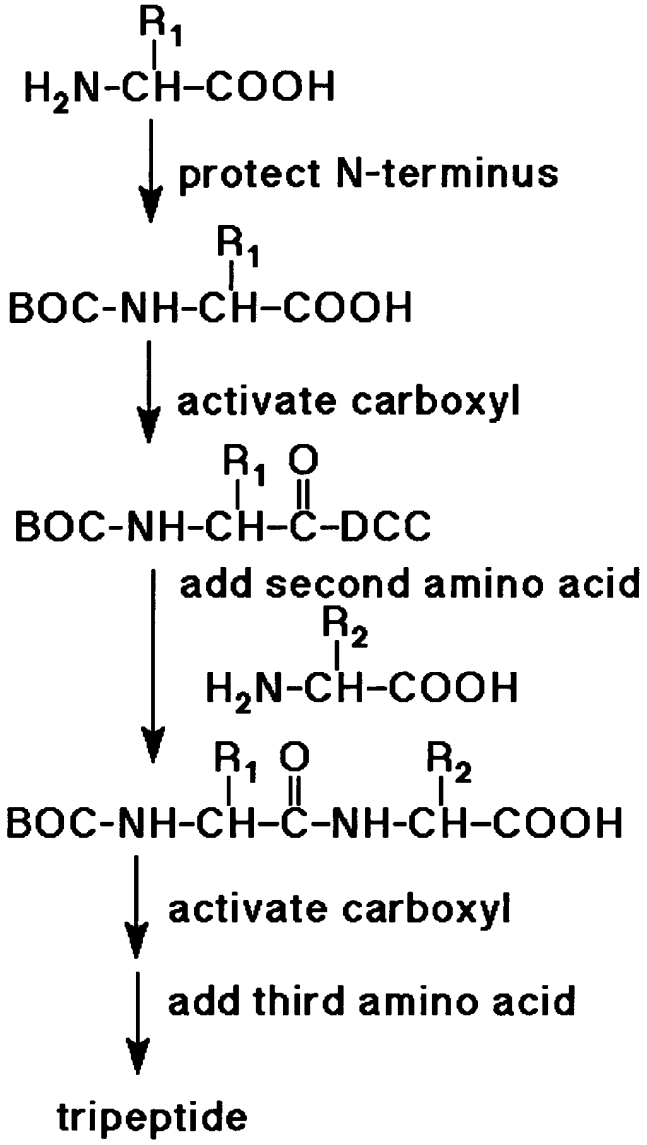
Peptide Sequencing

- **complete sequencing of a peptide can be done for up to about 20 amino acids in a row**
- **Edman degradation: selective cleavage of one amino acid off the N-terminus, followed by analysis to see what amino acid was removed**
  (the selective reagent is phenyl isothiocyanate, and the clipped-off product is a phenylthiohydantoin)

Peptide Synthesis

- **complete synthesis of peptides was started by Emil Fischer**
- **the procedure now can be automated up to about 100 amino acids**
- **protecting groups are essential to control the coupling reactions**

- **protect the N-side of AA1**
  (e.g., as a BOC-amide - easily removed later)
- **activate the C-side of AA1**
  (e.g., with DCC - milder than $SOCl_2$)
- **couple active AA1 to AA2**
  to form a dipeptide (AA2-AA1)
  (note that the N-terminus is still protected)
- **activate the free carboxyl group of the dipeptide, add AA3**
  (forms the tripeptide, AA1-AA2-AA3)
- **repeat for each amino acid of the polypeptide**
- **eventually remove the protecting group from the N-terminus**



Proteins - Natural Polypeptides

- **protein classifications may be based on structure or function or origin**
- **proteins are always a polypeptide (sometimes more than one chain) and may also have other types of molecules associated**

Conjugated Proteins are asociated with other types of molecules

- **glycoproteins - sugars**
- **lipoproteins - lipids (nonpolar fats and oils)**
- **nucleoproteins - nucleic acids**
- **heme proteins - porphyrin molecule**
- **metalloproteins - metal ions (often in a heme group)**

Protein Shapes

- **fibrous proteins - side-by-side polypeptide chains**
  usually water-insoluble, mechanically strong
  useful for structure, muscle
- **globular proteins - compact, variable shapes**
  usually water-soluble, transported around the body
  useful for specific functions like catalysis (enzymes)

Protein Functions

- **enzymes - catalysts for controlling rates of specific reactions**
- **hormones - regulators of specific body processes**
- **transport - control of movement of other molecules or ions**
- **structure - muscle, skin, etc.**
- **storage - nutrient storage**
- **protection - antibodies**

Protein Structure - Primary Structure

- **amino acid sequence**
- **determination of the specific order of amino acid residues**

Protein Secondary Structure

- **regular conformations of the peptide chain**
  - **alpha-helix - a coil held together by H-bonding**
  - **beta-sheet (or pleated sheet) - antiparallel sections of peptide chains held together by H-bonding**

Protein Tertiary Structure

- **complete 3-dimensional structure of the protein**
- **includes disulfide bridges, ionic interactions, H-bonding, other polar interactions and nonpolar (hydrophobic) interactions**

Protein Quaternary Structure

- **aggregation of several peptides chains into a larger protein unit**

Enzymes - Protein Catalysts

- **enzymes affect the rate of a particular reaction**
  usually very specific as to what reaction or family of reactions will be catalyzed
- **nomenclature: -ase suffix after a description of the reaction they catalyze**
  - **hydrolase - hydrolysis**
  - **isomerase - isomerization**
  - **transferase - transfer of a group from one molecule to another**

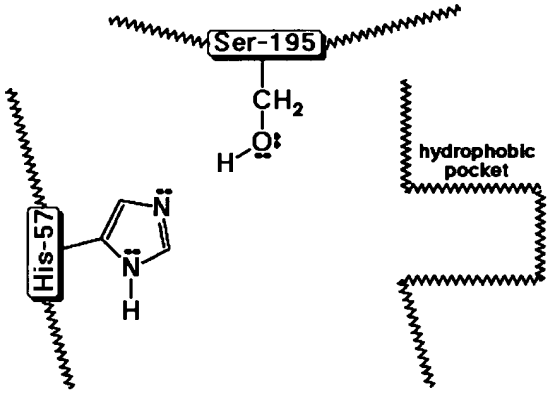
- lyase - elimination or addition of a small molecule, like water
- oxidoreductase - oxidation or reduction (usually with NADH or similar cofactor)
- ligase - binding of two molecules (usually with ATP cofactor)

- **note that enzymes can catalyze a reaction in both directions**
  like any catalyst, they provide a lower-energy pathway between reactants and products
  actual direction depends on the concentrations of substrates and cofactors

Chymotrypsin - A Specific Example of a Proteolytic Enzyme

- **chymotrypsin cleaves an amide bond next to an aromatic amino acid**
- **the imidazole ring of a nearby histidine specifically adds or removes a proton to aid in nucleophilic attack or leaving group departure**

The Chymotrypsin Active Site



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Pharmaceutical Patents

Title: Angiopoietins and methods of treating hypertension
United States Patent: 7,052,695
Issued: May 30, 2006
Inventors: Kalish; Susan Croll (Tarrytown, NY)
Assignee: Regeneron Pharmaceuticals, Inc. (Tarrytown, NY)
Appl. No.: 274222
Filed: October 18, 2002

Abstract

The invention generally relates to angiogenic factors and more particularly to the angiopoietin family of growth factors and to methods of using these growth factors to induce vasodilation and hypotension and reducing hypertension.

SUMMARY OF THE INVENTION

The invention generally relates to angiogenic factors and more particularly to the angiopoletin family of growth factors and to methods of using these growth factors to induce vasodilation.

One preferred embodiment of the method of the invention is a method of inducing vasodilation in a mammal comprising administering to the mammal an TIE2 receptor activator capable of inducing vasodilation.

Also preferred is a method of increasing blood flow to ischemic tissue in a mammal comprising administering to the mammal an TIE2 receptor activator capable of increasing blood flow to ischemic tissue.

Another preferred embodiment is one wherein the ischemic tissue is cardiac tissue, hepatic tissue, renal tissue, or skeletal muscle tissue.

Yet another preferred embodiment of the invention is a method of inducing hypotension in a mammal comprising administering to the mammal a TIE2 receptor activator capable of inducing hypotension.

Also preferred is a method of attenuating acute hypertension in a mammal comprising administering to the mammal a TIE2 receptor activator capable of attenuating acute hypertension.

Still another preferred embodiment of the invention is a method of treating vascular insufficiency in a mammal comprising administering to the mammal a TIE2 receptor activator capable of treating vascular insufficiency.

Another preferred embodiment is a method wherein the vascular insufficiency is penile erectile dysfunction, Raynaud's Syndrome, or diabetic vascular insufficiency.

Also preferred is a method of attenuating chronic hypertension in a mammal comprising administering to the mammal a TIE2 receptor activator capable of attenuating chronic hypertension.

One preferred embodiment is wherein the chronic hypertension is pulmonary hypertension including primary pulmonary hypertension and secondary pulmonary hypertension.

Another preferred embodiment is one in which the TIE2 receptor activator is Ang-1, Ang-1*, or Ang1-FD-Fc-FD; a small molecule; an activating antibody or a fragment thereof, including a scFv fragment of an antibody; and wherein the antibody is a monoclonal antibody.

A preferred embodiment of the invention is one wherein the mammal is a human.

DETAILED DESCRIPTION OF THE INVENTION

As described in greater detail below, Applicant has invented a method of inducing vasodilation in a mammal comprising administering to the mammal a TIE2 receptor activator. In one preferred embodiment of the invention, the mammal is a human and the TIE2 receptor activator is Ang1, Ang1*, or Ang1-FD-Fc-FD.

The invention further provides for a method wherein the TIE2 receptor activator is Ang1, or a fragment or derivative thereof capable of activating the TIE2 receptor.

The invention also provides for a method wherein the TIE-2 receptor activator is an activating antibody, or a fragment or derivative thereof capable of activating the TIE-2 receptor, including a single chain Fv (scFv).

The invention further provides for a method wherein the TIE-2 receptor activator is a small molecule, or a fragment or derivative thereof capable of activating the TIE2 receptor.

By way of example, but not limitation, the method of the invention may be useful in treating clinical conditions that are characterized by hypertension, including pulmonary

hypertension, and ischemic conditions including chronic ischemias such as diabetic ischemia, Bueger's Syndrome and Raynaud's Syndrome, and acute ischemias such as those associated with myocardial infarction and stroke. Other clinical applications include treatment of penile erectile dysfunction associated with decreased blood flow.

The present invention comprises TIE2 ligands such as, for example, Ang1, Ang1*, and Ang1-FD-Fc-FD as well as their **amino acid** sequence and also functionally equivalent molecules in which **amino acid** residues are substituted for residues within the sequence resulting in a silent change. For example, one or more **amino acid** residues within the sequence can be substituted by another **amino acid**(s) of a similar polarity that acts as a functional equivalent, resulting in a silent alteration. Substitutes for an **amino acid** within the sequence may be selected from other members of the class to which the **amino acid** belongs. For example, the class of nonpolar (hydrophobic) **amino** acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar **neutral amino** acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (**basic**) **amino** acids include arginine, lysine and histidine. The negatively charged (**acidic**) **amino** acids include aspartic **acid** and glutamic **acid**. Also included within the scope of the invention are proteins or fragments or derivatives thereof which exhibit the same or similar biological activity and derivatives which are differentially modified during or after translation, e.g., by glycosylation, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc.

Antibodies, including monoclonal antibodies, that activate the TIE2 receptor are also contemplated by the invention. For preparation of monoclonal antibodies, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495 497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in "Monoclonal Antibodies and Cancer Therapy," Alan R. Liss, Inc. pp. 77 96) and the like are within the scope of the present invention.

The monoclonal antibodies may be human monoclonal antibodies or chimeric human-mouse (or other species) monoclonal antibodies. Human monoclonal antibodies may be made by any of numerous techniques known in the art (e.g., Teng et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:7308 7312; Kozbor et al., 1983, Immunology Today 4:72 79; Olsson et al., 1982, Meth. Enzymol. 92:3 16). Chimeric antibody molecules may be prepared containing a mouse antigen-binding domain with human constant regions (Morrison et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:6851, Takeda et al., 1985, Nature 314:452).

Various procedures known in the art may be used for the production of polyclonal antibodies. For the production of TIE2 activating antibodies, various host animals, including but not limited to rabbits, mice and rats can be immunized by injection with TIE2 receptor extracellular domain, or a fragment or derivative thereof. Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (Bacille Calmette-Guerin) and Corynebacterium parvum.

A molecular clone of an antibody to a selected epitope can be prepared by known techniques. Recombinant DNA methodology (see e.g., Maniatis et al., 1982, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.) may be used to construct nucleic **acid** sequences which encode a monoclonal antibody molecule, or antigen-binding region thereof.

The present invention provides for antibody molecules as well as fragments of such antibody molecules. Antibody fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab').sub.2 fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the F(ab').sub.2 fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent. Antibody molecules may be purified by known techniques, e.g., immunoabsorption or immunoaffinity chromatography, chromatographic methods such as HPLC (high performance liquid chromatography), or a combination thereof.

The method of the invention also contemplates the use of antibody fragments directed against Tie-2 called single chain Fvs. A single chain Fv (scFv) is a truncated Fab having only the V region of an antibody heavy chain linked by a stretch of synthetic peptide to a V region of an antibody light chain. See, for example, U.S. Pat. Nos. 5,565,332; 5,733,743; 5,837,242; 5,858,657; and 5,871,907 assigned to Cambridge Antibody Technology Limited incorporated by reference herein.

Ang1, Ang1*, or Ang1-FD-Fc-FD **pharmaceutical** compositions can be prepared. Ang1, Ang1*, or Ang1-FD-Fc-FD-containing **pharmaceutical** compositions typically include a therapeutically effective amount of Ang1, Ang1*, or Ang1-FD-Fc-FD combined with one or more pharmaceutically and physiologically acceptable **formulation** components selected for suitability with the mode of administration. Suitable **formulation** components include, but are not limited to, preservatives, diluting agents, emulsifying agents, suspending agents, solvents, fillers, bulking agents, buffers, delivery vehicles, excipients and/or **pharmaceutical** adjuvants. By way of non-limiting example, a suitable delivery vehicle may be water for injection or physiological saline solution. Buffered saline or saline mixed with serum albumin are other examples of suitable vehicles.

The vehicle solvent may be either aqueous or non-aqueous. In addition, the vehicle may contain other pharmaceutically acceptable components for maintaining the pH, osmolarity, viscosity, stability, etc. The vehicle may contain additional pharmaceutically acceptable components for affecting the rate of release of Ang1, Ang1*, or Ang1-FD-Fc-FD, or for promoting the absorption or penetration of Ang1, Ang1*, or Ang1-FD-Fc-FD.

Once the therapeutic composition has been formulated, it may be stored as a solution, suspension, gel, emulsion, solid, or dehydrated, or lyophilized powder. Such formulations may be stored either in a ready to use form or in a form requiring reconstitution or other manipulation prior to administration.

The optimal **pharmaceutical** formulations will be determined by skilled artisans. Such optimal formulations will depend upon, for example, route of administration and dosage. (See, for example, Remington's **Pharmaceutical** Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, Pa. 18042) pages 1435 1712, the disclosure of which is hereby incorporated by reference).

The **pharmaceutical** composition also may be formulated for slow-release or sustained circulation formulations. It is also contemplated that certain formulations may be administered orally. Ang1, Ang1*, or Ang1-FD-Fc-FD, which is administered orally, may be formulated as an elixir, tablet, capsule, or gel. The capsule may be designed to release the active portion of the **formulation** in the gastrointestinal tract when bioavailability is maximized and degradation is minimized. Additional components may be included to facilitate absorption of Ang1, Ang1*, or Ang1-FD-Fc-FD. Such components include, but are not limited to, diluents, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders.

The Ang1, Ang1*, or Ang1-FD-Fc-FD may be administered parenterally via a subcutaneous, intramuscular, intravenous, intraarterial, intranasal, intrapulmonary, or intraperitoneal route. Alternatively, Ang1, Ang1*, or Ang1-FD-Fc-FD may be administered orally, or into specific areas of the gastrointestinal tract, or via rectal, transdermal or topical routes. The frequency of dosing will depend on the pharmacokinetic parameters of the Ang1, Ang1*, or Ang1-FD-Fc-FD as formulated, and the route of administration used.

The specific dose may be calculated according to considerations of body weight, body surface area, or organ size. Further refinement of the calculations necessary to determine the appropriate dosage for treatment involving each of the above mentioned formulations and routes of administration is routinely made by those of ordinary skill in the art. Appropriate dosages may be determined through the use of established assays for determining dosages utilized in conjunction with appropriate dose-response data.

The final dosage regimen involved in a method for treating the above-described conditions will be determined by the attending physician, considering various factors which modify the action of drugs, e.g., the age, condition, body weight, sex and diet of the patient, the severity of the disorder or disease, time of administration, and other clinical factors. As studies are conducted, further information will emerge regarding the appropriate dosage levels for the treatment of the various diseases and conditions.

Toxicity of the compounds described herein can be determined by standard **pharmaceutical** procedures in cell cultures or experimental animals, e.g., by determining the LD.sub.50 (the dose lethal to 50% of the population) or the LD.sub.100 (the dose lethal to 100% of the population). The dose ratio between toxic and therapeutic effect is the therapeutic index. Compounds that exhibit high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a dosage range that is not toxic for use in humans. The dosage of the compounds described herein lies preferably within a range of circulating concentrations that include the effective dose with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact **formulation**, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See, e.g., Fingl et al., 1975, In: The Pharmacological Basis of Therapeutics, Ch.1, p.1).

It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity, or to organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administrated dose in the management of the clinical disorder of interest will vary with the severity of the condition to be treated and the route of administration. The severity of the condition may, for example, be evaluated, in part, by appropriate prognostic evaluation methods. Further, the dose and perhaps dose frequency, will also vary according to the age, body weight, and response of the individual patient.

In addition to conventional treatment modalities and routes of administration such as those described supra, numerous methods exist for genetically engineering mammalian cells. There is great interest in genetically engineering mammalian cells for several reasons including the need to produce large quantities of various polypeptides and the need to correct various genetic or other defects in the cells or in tissues and organs. The methods differ dramatically from one another with respect to such factors as efficiency, level of expression of foreign genes, and the efficiency of the entire genetic engineering process. Viral vectors are presently the most frequently used means for transforming cells and introducing DNA into the genome. In an indirect method, viral vectors, carrying new genetic information, are used to infect target cells removed from the body, and these cells are then reimplanted. Direct in vivo gene transfer into postnatal animals has been reported for formulations of DNA encapsulated in liposomes and DNA entrapped in

proteoliposomes containing vital envelope receptor proteins (Nicolau et al., Proc. Natl. Acad Sci USA 80:1068 1072 (1983); Kaneda et al., Science 243:375 378 (1989); Mannino et al., Biotechniques 6:682 690 (1988). Positive results have also been described with calcium phosphate co-precipitated DNA (Benvenisty and Reshef Proc. Natl. Acad Sci USA 83:9551 9555 (1986)). One method of genetically engineering mammalian cells that has proven to be particularly useful is by means of retroviral vectors. Retrovirus vectors and their uses are described in many publications including Mann, et al., Cell 33:153 159 (1983) and Cone and Mulligan, Proc. Natl. Acad. Sci. USA 81:6349 6353 (1984). Retroviral vectors are produced by genetically manipulating retroviruses. Retroviruses are RNA viruses; that is, the viral genome is RNA. This genomic RNA is, however, reverse transcribed into a DNA copy that is integrated stably and efficiently into the chromosomal DNA of transduced cells. Retroviral vectors are particularly useful for modifying mammalian cells because of the high efficiency with which the retroviral vectors "infect" target cells and integrate into the target cell genome. Additionally, retroviral vectors are highly useful because the vectors may be based on retroviruses that are capable of infecting mammalian cells from a wide variety of species and tissues. The ability of retroviral vectors to insert into the genome of mammalian cells have made them particularly promising candidates for use in the genetic therapy of genetic diseases in humans and animals.

Another commonly used viral vector system in the Adeno-associated virus (AAV). The broad host range, low incidence of immune response, and longevity of gene expression observed with this vector have enabled the initiation of several clinical trials using this gene delivery system. Another potential benefit of using AAV vectors is their ability to integrate in a site-specific manner when introduced in the presence of Rep proteins. In addition, adenoviral vectors are used in many experimental settings to mimic acute administration of a protein of interest in vivo for the purpose of studying biological effects of the protein.

Claim 1 of 5 Claims

1. A method of attenuating nitric oxide-mediated acute hypertension in a mammal comprising administering to the mammal an effective amount of a TIE2 receptor activator capable of attenuating acute hypertension, wherein the TIE2 receptor activator is Ang1-FD-Fc-FD.
